REMARKS

Regarding the status of all parent priority applications, the present application is a national stage filing of a PCT application No. PCT2003/027779 under 35 U.S.C. 371, that was filed on September 8, 2003.

The specification has been amended to address the trademark terminology referenced by the Examiner. A sentence has been inserted in Example 2 to provide more information on the BD TALON® resin which was used. The nature of the BD TALON® is not material to the practice of the invention. In addition, a web page on BD TALON® has been attached to the present response for your reference. The specification has also been amended to correct the reference to Figures 4 and 5 as identified by the Examiner.

Claims 1-12 and 21-23 have been canceled without prejudice or disclaimer as being drawn to a non-elected invention. Claims 13, 14 and 15 have been amended. Claim 20 has been canceled, and its substance is now included in claim 13. The application now includes claims 13-16 and 19. No new matter has been added.

Claims 14 and 15 which are objected under 37 C.F.R. 1.75(c) have been rewritten in the proper dependent form of a method claim. Claim 15 now includes the phrase "means for".

The claims at issue are specific for reducing tobacco specific nitrosamines (TSNAs) in air-cured tobacco plants. As explained in paragraph [0006], TSNAs in flue-cured tobacco have been resolved by venting combustion gases from curing barns; however, in air-cured tobacco varieties TSNA accumulation results from microbial activity. Prior research shows that misting tobacco leaves with ascorbic acid makes the TSNA situation worse (note a two-fold increase in TSNA levels relative to misting with water) , but imbibing the air cured leaves with ascorbic acid reduces the accumulation of TSNAs (see, Bush et al., "Formation of tobacco specific nitrosamines in air-cured tobacco", *55th Tobacco Science Research Conference-Recent Advances in Tobacco Science* 27:23-46 (2001). Further, a copy of the abstract and data from Rundlof, *J. Agric. Food Chem.* 48 (9) 438104388, 2000 (attached–referenced in paragraph [0006] of the application) shows that infiltration of leaves with ascorbic acid can reduce TSNAs. However,

as explained in paragraph [0006] treatment of a tobacco leaves to include imbibed or infiltrated ascorbic acid would be time consuming and expensive.

In this invention, it was determined that genetically engineering an tobacco to produced increased levels of endogenous levels of vitamin C ("ascorbic acid") results in substantially reduced TSNAs in air cured tobacco. Given the differences in results with spraying tobacco leaves and imbibing them, it was not known or obvious until this invention that endogenously producing higher levels of vitamin C would result in lower TSNAs in air cured tobacco. The results and methods of the claimed invention are superior to those achieved when tobacco leaves are treated with ascorbic acid. With reference to Example 5 (paragraphs [0052] to [0054], it can be seen that tobacco plants that are transformed with a gene in the vitamin C biosynthetic pathway produces higher amounts of endogenous ascorbic acid and has dramatically lower levels of TSNAs than non-genetically engineered plants at the end of the curing process (as such, the Examiner's conclusion that there is teaching of reducing TSNAs with genetically engineered tobacco with at least one gene in the vitamin C biosynthetic pathway is simply incorrect).

Claims 13-16, 19-20 as failing to comply with the written description requirement and as lacking enablement. These rejections are traversed.

Claim 13 is narrowly focused on air cured tobacco. In addition, claim 13 has been amended to specifically require that the genetically engineered tobacco which includes at least one gene from the vitamin C biosynthetic pathway (1) produces increased endogenous levels of vitamin C, and, has reduced levels of TSNAs upon completing said air curing step. A specific working example of the invention is provided in Example 5. Further, the application provides the sequence of a gene and amino acid not previously known to increase AsA content in tobacco leaves (claims 16 and 19 being specifically focused on this new pathway and are directed to its involvement in reducing TSNAs in air cured tobacco).

In short, the application demonstrates, for the first time, that genetically engineered tobacco with high endogenous levels of vitamin C results in low TSNAs in air cured tobacco. Given prior knowledge about AsA and its effect on TSNAs, coupled with the successful demonstration in the patent application that

incorporation of a gene into tobacco which is in the biosynthetic pathway for vitamin C to produce higher levels of endogenous vitamin C, it can be concluded that, contrary to the reasoning in the office action, almost any gene, now known or later discovered, which is engineered into air cured tobacco which results in increased endogenous levels of vitamin C (as now specifically required in claim 13) will have reduced levels of TSNAs upon completing the air curing step. Claim 13 is specific for a process which employs this finding in a process that involves air curing tobacco. Claim 16 and 19 are focused on the finding of a new gene in the vitamin C biosynthetic pathway being used in the claimed process. Given that the sequence is disclosed in the application, and the application demonstrates that high levels of endogenous vitamin C can be produced with this gene, these claims are specifically enabled by the patent application.

Claims 13-15 and 20 were rejected as being anticipated by Jain et al. This rejection is traversed. It is noted that the inventor of the present application is the corresponding author in the Jain reference. Nowhere in Jain is any reference made to lowering TSNAs in air cured tobacco. Only in the present application is the concept of air curing tobacco that is genetically engineered to produce high levels of endogenous vitamin C to achieve lower levels of TSNAs on air curing of tobacco taught. Claim 13 has been amended to specifically require air curing of genetically engineered tobacco, where the genetic engineering of the tobacco produces increased endogenous levels of vitamin C, and lower levels of TSNAs upon completing the air curing step (as is taught in Example 5 of the application). Given that there is no teaching in Jain concerning lowering TSNAs in air cured tobacco, this rejection should be withdrawn.

Claims 13-16 and 19-20 were rejected as being obvious over Jain in veiw of Arner. This rejection is traversed for essentially the same reason noted above. Arner, like Jain, does not describe MIOX being used in any way to lower TSNAs in air cured tobacco. Furthermore, Arner has no teaching that MIOX can be genetically engineered into tobacco to produce high levels of endogenous vitamin C. As such, no combination of Jain and Arner would make the claimed invention obvious.

The Bush reference, included in the information disclosure statement and discussed above, further highlights the unobviousness of the present invention. In

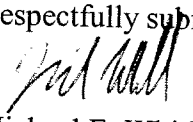
particular, on page 33 it is noted that "Results from these types of experiments are not readily repeatable...additional investigations are needed".

Claims 17 and 18 should be rejoined as these claims are directed to another gene in the vitamin C biosynthetic pathway being exploited in the claimed method.

In view of the foregoing, it is respectfully requested that the application be reconsidered, that claims 13-19 be allowed, and that the application passed to issue.

Should the Examiner find the application to be other than in condition for allowance, the Examiner is requested to contact the undersigned at the local telephone number listed below to discuss any other changes deemed necessary in a telephonic or personal interview.

Respectfully submitted,



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TALON™ His-Tag Purification Resins

Description

- *High **affinity** for polyhistidine-tagged proteins*
- *Resists metal leakage under denaturing conditions*
- *Performs well under a wide range of purification conditions*

BD TALON™ Metal **Affinity Resin** is a durable immobilized metal **affinity chromatography** (IMAC) **resin** that has a remarkable **affinity** and specificity for His-tagged proteins. **BD TALON™ Resin** is compatible with all commonly used IMAC reagents and allows protein purification under native or denaturing conditions. **BD TALON Resin** can be used in a variety of formats, including gravity-flow columns and small- or large-scale batches.

Reactive core contains cobalt

BD TALON Metal **Affinity Resin** uses cobalt ions for purifying recombinant polyhistidine-tagged proteins (1每3). The **BD TALON** reactive core, containing cobalt, has strict requirements for the spatial positioning of histidines. Only adjacent histidines or specially positioned, neighboring histidines are able to bind cobalt in this reactive core. In nickel-based resins (i.e., Ni-NTA **Resin**), these spatial requirements are less strict. Therefore, nickel-based resins are also able to bind histidines located in places other than the protein * s His-tag.

Uniform matrix

Cobalt-based resins have a more uniform structure than nickel-based resins. All reactive sites in **BD TALON Resin** look like three-dimensional pockets. In these pockets, cobalt is bound to three carboxyl groups and one nitrogen atom, and is able to bind to two other ligands, i.e., two histidines. In this configuration, cobalt is bound very tightly and does not leak out of the **resin**. Nickel-based resins are less homogeneous in structure because nickel ions can form two different coordination structures. One of them is a three dimensional pocket, similar to the **BD TALON™** ligand. The other structure is planar (flat). In this distorted, planar structure nickel is bound to only two carboxyl groups and one nitrogen atom. Since this binding is not very strong, planar reactive cores are not able to hold nickel ions as tightly, leading to leaking of the nickel ion from the **resin**. **BD TALON** Metal **Affinity Resin**, with its uniform matrix, provides high **affinity** binding in a variety of purification conditions, ensuring optimized protein purification.

BD TALON™ CellThru Resin is a novel IMAC (immobilized metal **affinity chromatography**) **resin** for purifying histidine-tagged proteins from crude cell lysates, sonicates, and fermentation harvests. This **resin** uses the same proprietary ligand as our **BD TALON** Metal **Affinity Resin**, but has larger beads (300每500 µm) permitting cellular debris to flow through without centrifugation. **BD TALON** CellThru **Resin** captures poly-histidine tagged protein directly from crude lysates in one quick step, minimizing protein degradation and generating higher yields of purified protein than conventional strategies. This **resin** consists of large agarose beads (300每500 µm) packed into standard **chromatography** columns with large-pore frits to prevent column blockage. Particulate material flows between the beads while the soluble protein product is captured by the ion exchange functional groups attached to the beads. **Resin** is ideal for use in expanded bed **chromatography** for recovering even higher amounts of protein and is recommended for applications such as high-throughput purification of Histidine-tagged proteins from crude extracts. We also offer CellThru 2-ml and 10-ml Disposable Columns with a filter pore size of 90每130 µm, allowing cellular debris to flow through easily.

BD TALON™ Superflow Resin is specifically designed for quick and effective purification of His-tagged proteins at high flow rates and medium pressure (up to 150 psi).

BD TALONspin™ Columns are ready-made spin columns containing **BD TALON**-NX **Resin** for the simultaneous purification of several His-tagged proteins in parallel in only 30 minutes. These convenient columns are recommended small-scale single-use applications such as verifying positive transformants for His-tagged protein expression levels, or trial-level purification protocols.

The **BD TALON™ Disposable Gravity Columns** are empty, disposable columns for single-use applications which simplify set-up as well as clean-up.

BD TALON™ xTractor Buffer Kit is an optimized buffer system for extraction of proteins from fresh or frozen bacterial cells. The **BD TALON** xTractor Buffer is useful in extracting proteins over a broad range of molecular weights, including high molecular weight proteins that cannot be extracted unless the membrane is fully disrupted. Lysozyme and DNase I are included to help disrupt membranes and to reduce solution viscosity, respectively. The **BD TALON** xTractor Buffer is compatible with all **BD TALON™** Resins, allowing quick purification of His-tagged proteins.

BD TALON™ Purification Kit is the ideal place to start when using **BD TALON resin** in your applications. This convenient kit contains **BD TALON Resin**, columns, and all the buffers necessary to extract, wash, and elute His-tagged proteins. Sufficient buffer is provided for 10 extractions of 1-g bacterial pellets.

BD TALON™ Buffer Kit is a supplemental kit containing concentrated forms of optimized buffers for extracting, washing, and eluting proteins.

BD TALON™ HT 96-Well Plate is designed for high-throughput protein purification system of up to 96 His-tagged proteins in 30 minutes at up to 1 µg protein/well; each well of the filtration plate is pre-loaded with sufficient **BD TALON** Superflow **Resin** to bind up to 1 mg of histidine-tagged protein. The plate purification is compatible with either a vacuum manifold (such as the NucleoVac Vacuum Manifold, Cat. No. 636030), or a centrifuge that has a rotor for 96-well plates. The HT 96-well Plate is provided with convenient top and bottom seals for shipping and storage, as well as a Collection Plate that can be used for receiving the eluted his-tagged proteins and storage of the samples.

Applications
- Poly His-tagged protein purification
- Recombinant protein purification & identification
- Batch scale protein purification
- FPLC purification
- Gravity flow

Contents
BD TALON™ Purification Kit Components
Equilibration Buffer
Equilibration/Wash Buffer
Elution Buffer
BD TALON Metal **Affinity Resin**
2-ml Disposable Columns
10-ml Disposable Column
User Manual (PT1320-1)

BD TALON™ xTractor Buffer Kit Components
200 ml xTractor Buffer
DNase I

Lysozyme

Preparation and Storage

4 ~ C for TALON **Resin**, **BD TALON**™ CellThru **Resin**, **BD TALON**™ Superflow **Resin** & BD TALONspin™ Columns
Room temperature for **BD TALON** Disposable Columns & Buffers

References

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2. Porath, J. (1992) *Protein Express. Purif.* **3**:263每281.
3. Yang, T., *et al.* (1997) *Amer. Biotechnol. Lab.*, pp. 12每14.
4. Protein Purification Products Overview (2001) *Clontechniques* **XVI**(1):27每32.